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THE ACCESSORY CELL AND YOLK HALO OF THE OÖCYTE OF THE FRESHWATER TURBELLARIAN *HYDROLIMAX GRISEA* (PLATYHELMINTHES; PLAGIOSTOMIDAE) [1]

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Developing eggs are often associated with cells which, when numerous, form cell layers around the ovum, or, when single or few in number, appear to attach to the ovum in some way (Wilson, 1925). It is generally assumed that cells associated with eggs provide nutriment or act as nutrimental intermediaries to the growing oöcyte (Raven, 1961). In the turbellarian *Hydrolimax grisea* a single cell becomes intimately associated with the egg: this accessory cell completely surrounds the growing oöcyte and remains with the developing egg throughout its growth period (Newton, 1970). At the time of fertilization, the accessory cell remains in place around the egg—the sperm must penetrate the accessory cell before reaching the egg (Newton, 1970). Surrounding the oöcyte and its investing accessory cell in *Hydrolimax* is the yolk halo (Hyman, 1938; Newton, 1970). In histological preparations, the yolk halo consists of two layers, the inner of fine radial fibers and the outer of spherules. Hyman (1938; page 16) concluded the yolk halo to be ". . . a non-cellular halo presumably of nutritive nature." There is no evidence of utilization of the yolk halo material by the growing oöcyte or by the accessory cell (Newton, 1970).

Professor Ulric Dahlgren, of Princeton University, first observed the accessory cell and yolk halo of the oöcyte of *Hydrolimax* (Hyman, 1938). Opportunity for the present author to study some of Dahlgren's research notes and histological preparations of *Hydrolimax grisea* was made possible by a loan of the material from Dr. Ernst Kirsteur of the Department of Living Invertebrates of the American Museum of Natural History. The observations of Dahlgren (unpublished), Hyman (1938) and Newton (1970) are supplemented in the present report by phase-contrast and electron-microscopic studies of the accessory cell and yolk halo.

Materials and Methods

The material for this study was obtained from sources previously reported (Newton, 1970). Wet-mount squash preparations of live material were studied and photographed with a Zeiss Photomicroscope using phase optics.

For electron microscopy, specimens of *Hydrolimax* were fixed whole, initially at room temperature or on ice, and subsequently diced with a razor blade in fixative on ice. The material was fixed for two hours in 3% glutaraldehyde in 0.1 M

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sodium cacodylate to which a trace of calcium chloride had been added, pH 7.1 (adjusted with HCl), followed by post-fixation in 2% osmium tetroxide, according to a procedure devised by D. P. Costello (personal communication). The material was dehydrated in a graded series of ethanol, passed through propylene oxide and embedded in Epon-Araldite. Sections were cut on a Sorvall Porter-Blum MT-2 ultra-microtome with glass or diamond knives and stained with 3% uranyl acetate (aqueous) alone or in combination with 0.5% lead citrate. The sectioned material was studied with a Zeiss 9S2 electron microscope.

Observations

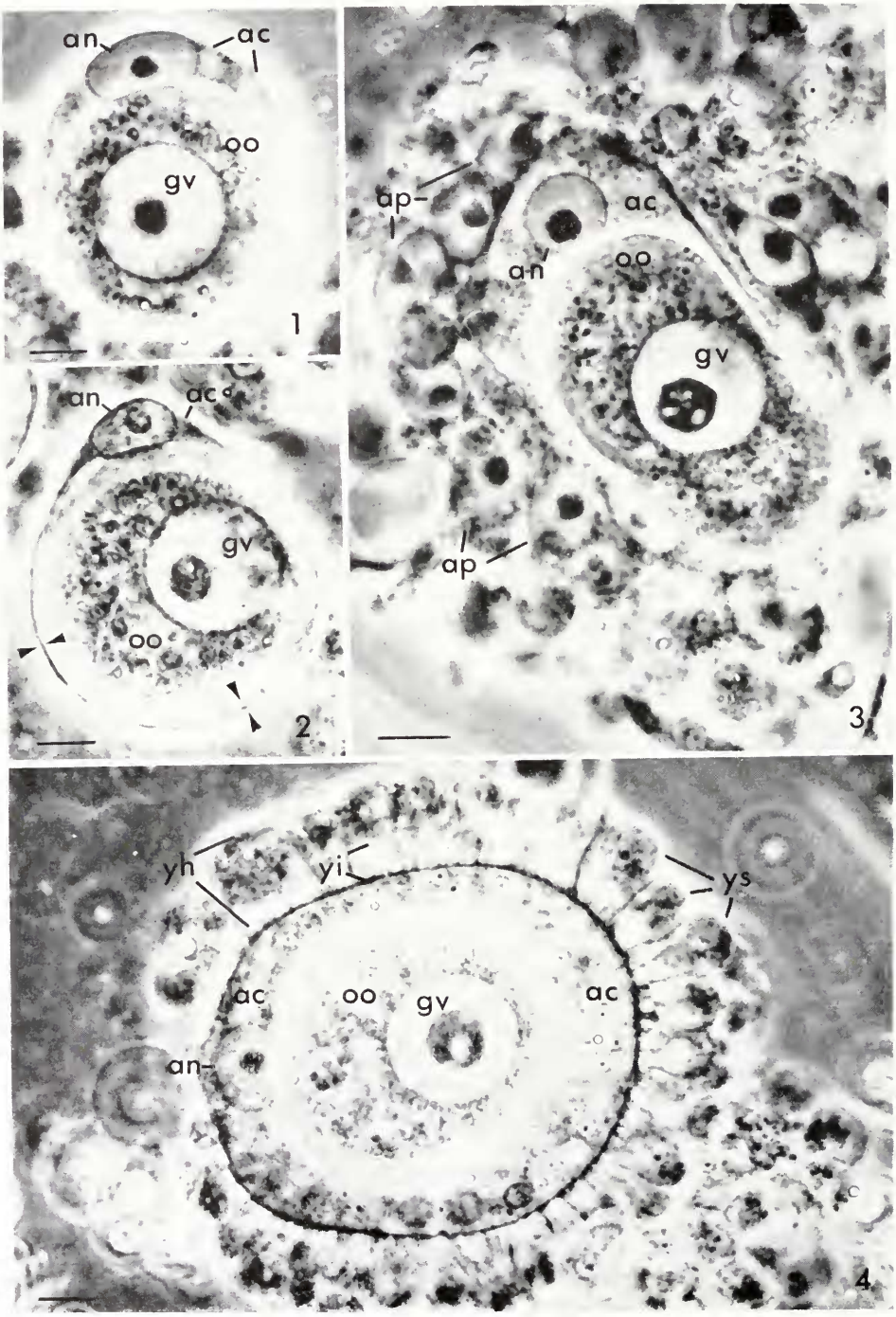
In the life cycle of *Hydrolimax,* female germ cells first appear in the parenchyma of worms collected in early fall of each year. Oögonia occur singly, or, rarely, in groups of three or more, not united by cell connectives or processes; they are free of association with other cells of the turbellarian parenchyma. Later many small, young oöcytes are found free of cell associations in squash preparations of living material.

The growing oöcyte becomes closely associated with cells of the parenchyma—the accessory parenchymal cells (Newton, 1970; his Figs. 3, 6). One of the accessory parenchymal cells becomes the accessory cell. Newton's Figure 7 shows an accessory cell fixed in the process of enveloping an oöcyte. By the mid-Fall of each year, the growing oöcytes of *H. grisea* have each been enveloped by an accessory cell. The chronology of the association of oöcyte and accessory cell can be correlated with cytological events of oögenesis: the accessory cell completely surrounds the young oöcyte after the dissolution of the chromosome bouquet (diplotene) prior to the extensive growth of the oöcyte.

Figure 1 is a phase-contrast micrograph of an oöcyte and its accessory cell. Although the phase halo in this micrograph makes it difficult to discern, the accessory cell has completely enveloped the oöcyte. Careful study through many planes of focus showed that the cytoplasm of the accessory cell is very thin on the side diametrically opposite the accessory cell nucleus. It may be significant to note that in squash preparations of living material accessory cells have not been observed *in the process* of enveloping oöcytes. The process of envelopment may be fairly rapid. The squashing of living turbellarians disrupts the parenchyma and apparently prevents normal accessory cell-oöcyte contact.

The oöcyte in Figure 2 is enveloped for the most part by a thin layer of accessory cell cytoplasm. In the vicinity of the accessory cell nucleus, the cytoplasm is thicker. Apparently the enveloping layer of accessory cell cytoplasm becomes attenuated by the relatively rapid growth of the oöcyte. Eventually, however, the accessory cell grows, for in later stages the layer of accessory cell cytoplasm surrounding the growing oöcyte thickens (Figs. 3, 4) (*cf.* Newton, 1970).

In squash preparations of live turbellarians collected in the late fall, the accessory cell, with its enveloped oöcyte, is surrounded by many accessory parenchymal cells (Fig. 3). The number of accessory parenchymal cells increases. By mid-Winter the accessory parenchymal cells have *apparently* been replaced by the spherules of the yolk halo (Fig. 4) (*cf.* Newton, 1970). The accessory parenchy-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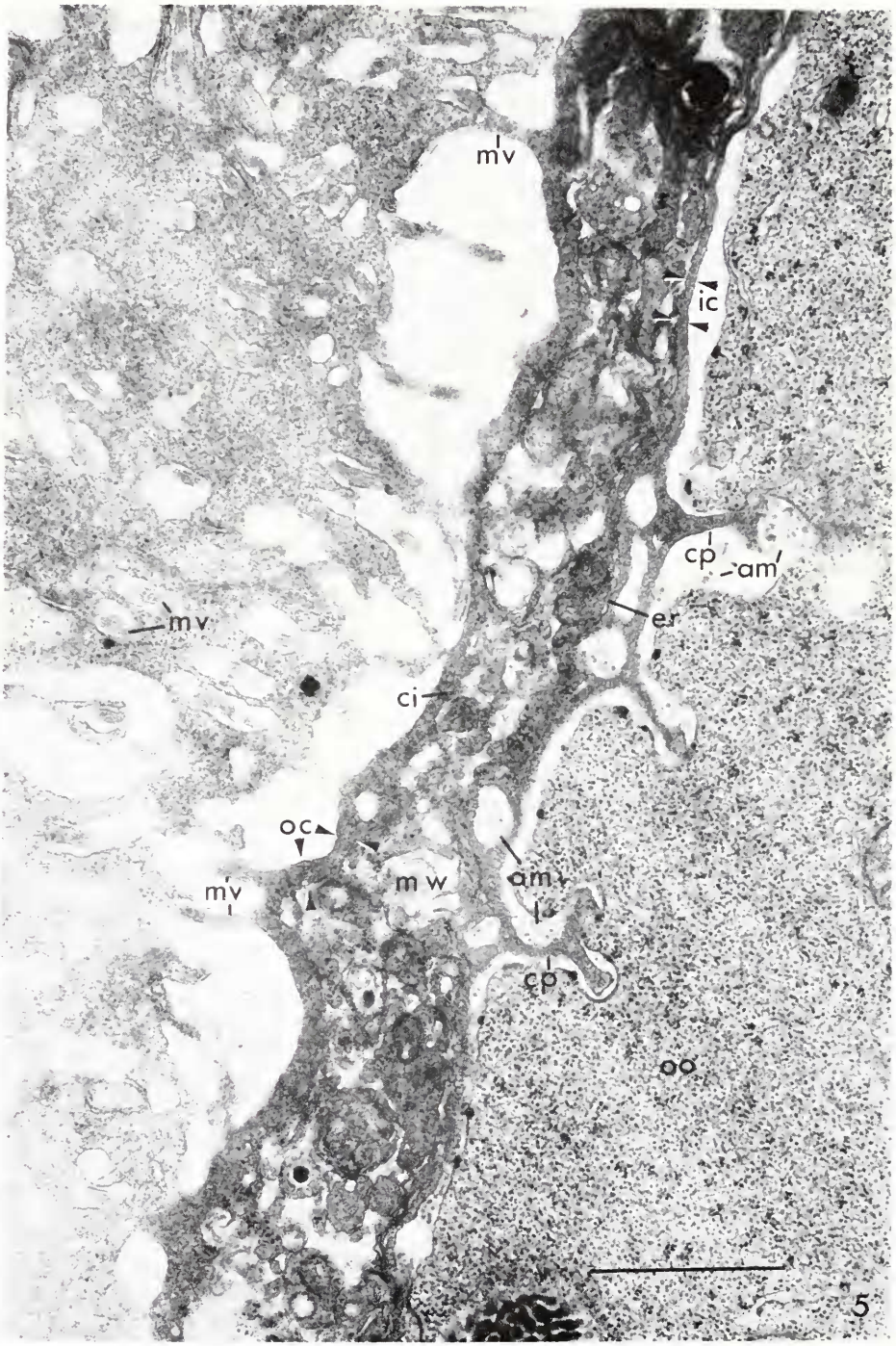
mal cells, however, become transformed into the spherules of the outer portion of the yolk halo (Fig. 4).

The accessory cell is in close apposition to the oöcyte (Figs. 1–5). In some specimens fixed, sectioned and stained for light microscopy, a wide space exists between the inner cell boundary of the accessory cell and the surface of the oöcyte (Newton, 1970; his Figs. 8, 12). The space is an artifact of fixation caused by shrinkage of the oöcyte, for no space is seen between accessory cell and oöcyte in living material studied by phase optics. A shrinkage space exists between oöcyte and accessory cell in some specimens fixed and sectioned for electron microscopy. Generally, however, electron micrographs show the accessory cell to be separated from the oöcyte surface by a distance no greater than 0.2 μ (Fig. 5). Cytoplasmic processes from the inner surface of the accessory cell penetrate the oöcyte no deeper than 0.8 μ (Figs. 5, 6, 7). The cytoplasm of the accessory cell and the cytoplasm of the oöcyte do not appear to intermix. The membranes of the two cells remain intact. The tips of the accessory-cell projections often touch the surface of the oöcyte (Fig. 6) and prehaps fuse with the cell membrane of the oöcyte, as indicated in Figure 6. The space between oöcyte and accessory cell is empty in specimens prepared for light microscopy. In material prepared for electron microscopy amorphous particles of medium electron density are found between oöcyte and accessory cell (Figs. 5, 6, 7). Similar amorphous particles are located within membrane-bound vesicles of the accessory cell (Figs. 5, 6, 7). An open vesicle in Figure 6 suggests an interchange of amorphous particles between accessory cell and the intercellular space. In sections stained with uranyl acetate or with uranyl acetate in combination with lead citrate, electron-dense bodies of unknown significance are located on or immediately beneath the plasma membrane (Figs. 5, 7, and especially 6) of the oöcyte.

Sections through accessory cells show that the cytoplasm immediately beneath the outer and inner membranes is uniformly compact and generally devoid of organelles and inclusions (Figs. 6, 7). The layers of relatively homogeneous cytoplasm at the surfaces of the accessory cell are here called the outer cortex and the inner cortex. The cortices are approximately 730 Å thick. The cortical cytoplasms are granular, consisting of dark particles, approximately 100 Å or less, in a ground substance of medium electron density (Figs. 6, 7). Between the cortices are all manner of membranous elements and some granular components (Figs. 5–9). Mitochondria are the only distinctive organelles. Whorls of membrane, membranous cisternae of irregular shape containing wisps of electron-dense material, and endoplasmic reticulum are the major components of inter-cortical cytoplasm.

Microvilli arise from the outer surface of the accessory cell (Figs. 5, 7) and often project 3 to 4 μ into cells of the turbellarian parenchyma (Figs. 5, 8). The

FIGURES 1–4. The events depicted in this plate occur over a five- to six-month period. The young oöcyte is surrounded by an accessory cell (Fig. 1) before oöcyte growth (Fig. 2). Accessory parenchymal cells become associated with the outer surface of the accessory cell (Fig. 3) and transform into the spherules of the outer portion of the yolk halo (Fig. 4); phase-contrast micrographs; Figures 1, 2, 4, 784×; Figure 3, 980×; ac, accessory cell; an, accessory cell nucleus; ap, accessory parenchymal cell; gv, germinal vesicle (nucleus) of oöcyte; oo, oöcyte; yh, yolk halo; yi, inner portion of yolk halo; ys, spherules of outer portion of yolk halo; scale = 10 μ.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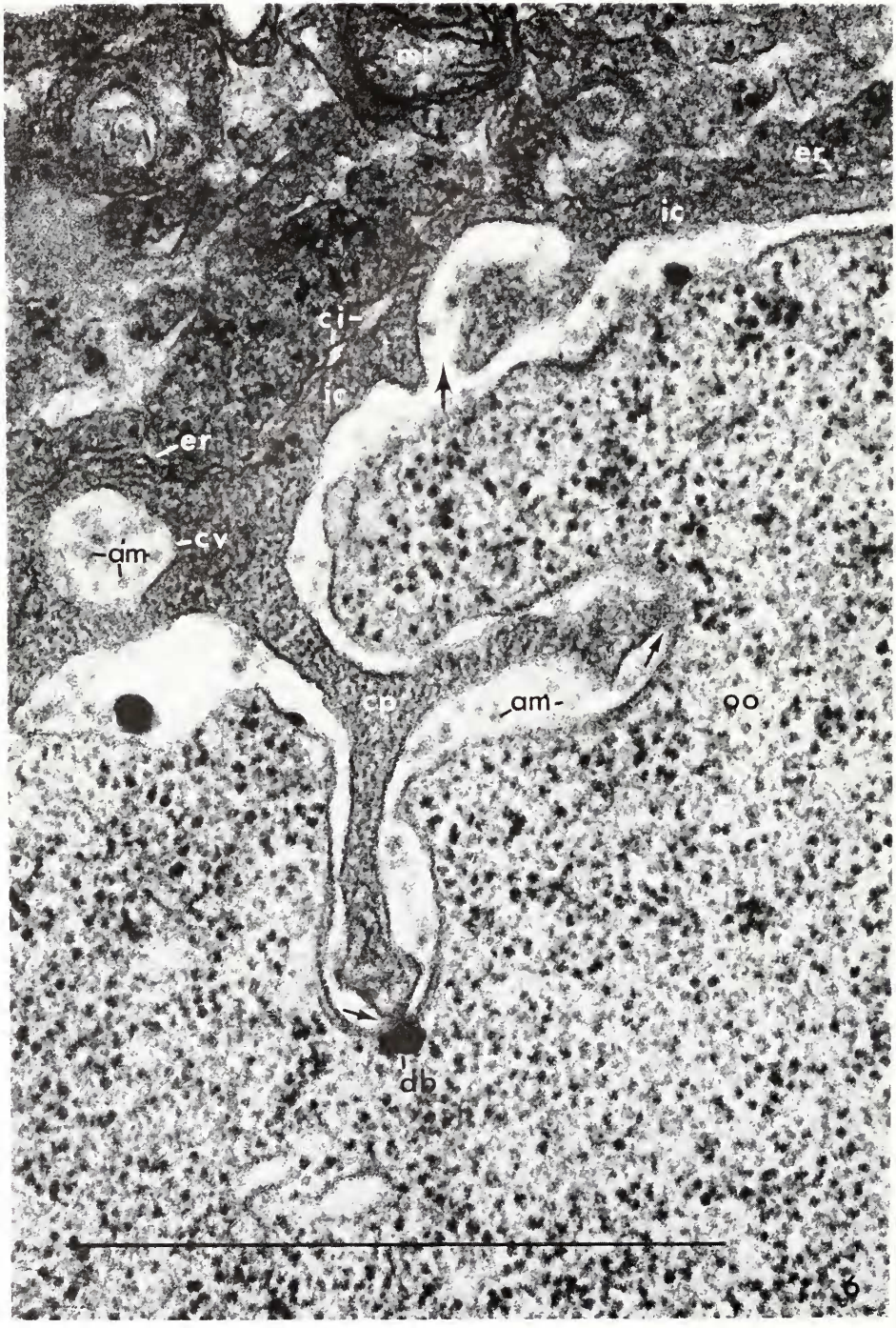
microvilli of the accessory cell do not fuse with adjacent cells but occupy membrane-delimited canals within the cytoplasm of adjacent cells (Figs. 5, 8). The appearance by light microscopy of the inner portion of the yolk halo, consisting of fine radial fibers (Fig. 4) (Newton, 1970; his Fig. 17), is explained by the numerous microvilli which extend from the accessory cell. Electron-dense filaments are oriented longitudinally within microvilli (Figs. 7, 9); no filaments are observed in the projections from the inner surface of the accessory cell into the oöcyte (*cf.* Figs. 5, 6, 7). Four to eight filaments are observed in transverse profile of microvilli (Fig. 9).

Evidence for the transformation of accessory parenchymal cells into the outer layer of the yolk halo was first obtained by the present author from some of the slides of Dahlgren. In Dahlgren's material, fixed with hot Flemming's fluid and stained with an undesignated hematoxylin, a basophilic material is present in the spherules of the outer layer of the yolk halo; this material is not bounded by a nuclear membrane. Dahlgren suggested in his research notes that the nucleus of the accessory parenchymal cell may be degenerating in the process of transformation by the accessory parenchymal cell into a yolk-halo spherule. In material prepared for light microscopy in Newton's (1970) study, evidence for nuclear degeneration is lacking: no basophilia is seen in the yolk spherules. Newton (1970; his Fig. 12) suggested that the yolk halo may be secreted by the accessory parenchymal cell. The discrepancy between Dahlgren's observations and those of Newton's study is probably a result of the use of different methods of fixation and staining. Evidence that Dahlgren's interpretation is correct comes from electron micrographs of the outer layer of the yolk halo (Figs. 8, 10). The spherules of the yolk halo are composed of organelles, primarily vesicles, but also endoplasmic reticulum, a few scattered mitochondria, some microtubules, all bounded by a cell membrane, and without nuclei. After Feulgen-Fast Green the yolk halo spherules are Feulgen-negative. The vesicles (Fig. 10) are conspicuous because of the homogeneous, electron-dense material unevenly distributed within them. Transformation of accessory parenchymal cells into the spherules of the outer portion of the yolk halo remains an enigma: the function of the spherules has not been ascertained. Evidence for the utilization of the yolk-halo spherules by the oöcyte is lacking.

Discussion

According to the system of classification of eggs proposed by Korschelt and Heider (1902; cited in Wilson, 1925; and Raven, 1961), the egg of *Hydrolimax* is of the nutrimental type and its accessory cell is a nurse cell. The accessory cell

Figure 5. The relationship between accessory cell and oöcyte is marked by the cytoplasmic processes extending from the inner surface of the accessory cell into the developing egg. Microvilli extend from the outer surface of accessory cell into membrane delimited canals in cells of the turbellarian parenchyma; uranyl acetate; 26,871×; am, amorphous particles; ci, swollen cisternae of endoplasmic reticulum; cp, cytoplasmic process; er, endoplasmic reticulum; ic, inner cortex; mw, membranous whorl; mv, microvillus; oc, outer cortex; scale = 1 μ.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of *Hydrolimax* is unique among the plagiostomids (Hyman, 1938) and apparently unique among the animal phyla.

The relationship between accessory cell and oöcyte in *Hydrolimax* is one of close apposition of the cells involved, and of cytoplasmic projections into the oöplasm from the inner surface of the accessory cell. There is no evidence that the cytoplasms of the accessory cell and of the oöcyte intermingle. Gondos (1970) observed close apposition of cell membranes of germ cells and granulosa cells, and the presence of interdigitating cytoplasmic projections from granulosa cells into oöcytes, of young rabbits. In the human, "localized projections from both the oöcyte and the follicle cells interdigitate in the intercellular space and appear to reflect a constant active interchange between the oöcyte and its follicle wall" (Hertig and Adams, 1967; page 668). The extension of accessory cell processes into the oöcyte in *Hydrolimax* may likewise reflect an interchange between the two cells.

Microvilli extend from the outer surface of the accessory cell, into extracellular spaces or into membrane-delimited canals of adjacent parenchymal or oviducal cells. Since microvilli are generally present on cells which have absorption as a primary function (Bloom and Fawcett, 1968), the function of the outer surface of the accessory cell may be to absorb nutrient material for transport across the accessory cell to the growing oöcyte. The speculation is amenable to simple experimental analysis, as the oöcyte and its surrounding accessory cell are easily isolated from the parenchyma of young turbellarians.

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Summary

1. A single cell, the accessory cell, completely surrounds the growing oöcyte of *H. grisea*.

2. Cytoplasmic processes from the inner surface of the accessory cell extend into the oöcyte. The cytoplasms of the accessory cell and of the oöcyte do not intermix.

3. Microvilli extend from the outer surface of the accessory cell.

4. It is suggested that the accessory cell is a nurse cell which transports nutrient material to the growing oöcyte.

FIGURE 6. Small arrows indicate apparent contact of tips of cytoplasmic process with oöcyte membrane. Large arrow points to opening of a cytoplasmic vesicle of accessory cell into intercellular space; uranyl acetate, 91,140×; cv, cytoplasmic vesicle; db, dense body; mi, mitochondrion; scale = 1 μ.

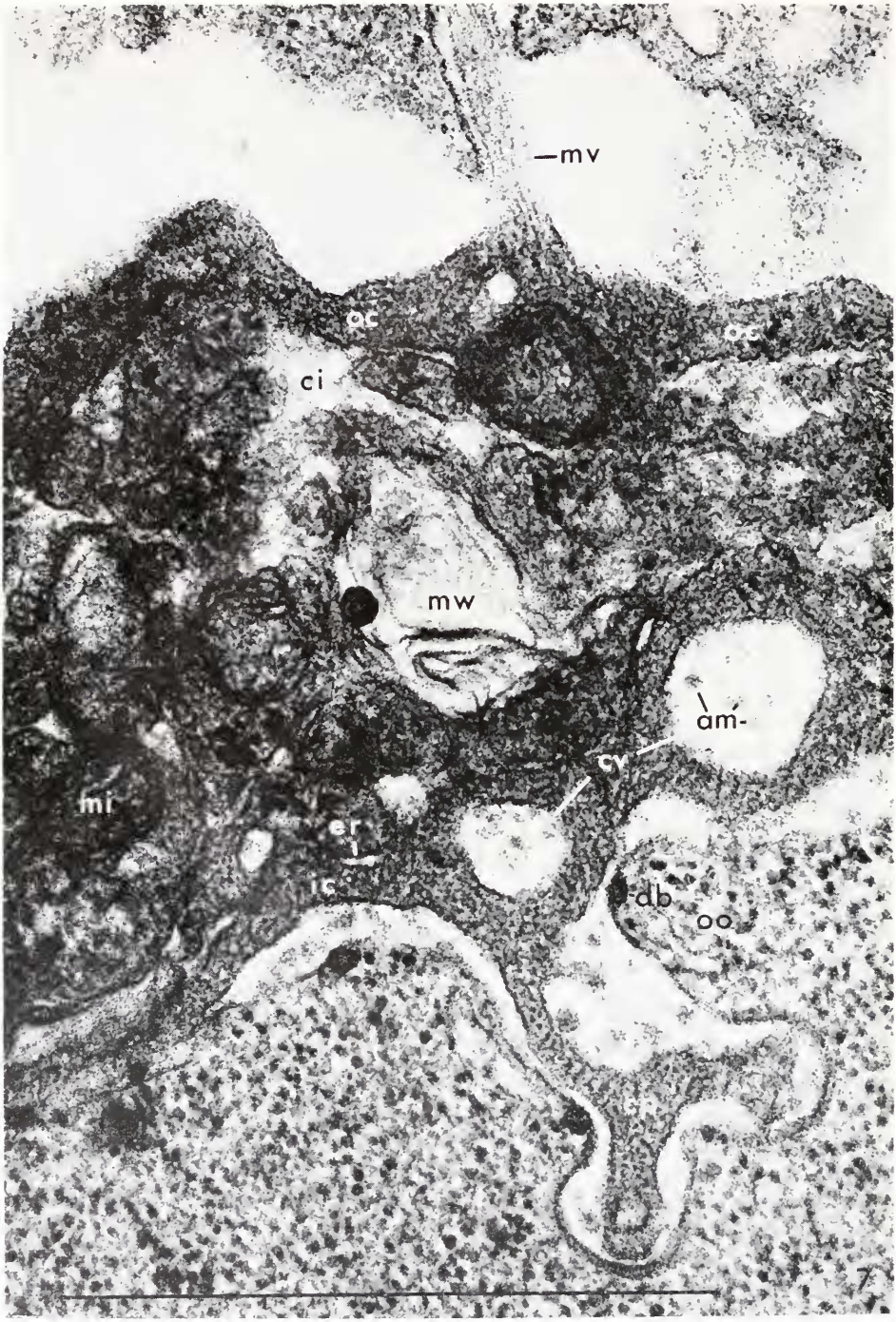
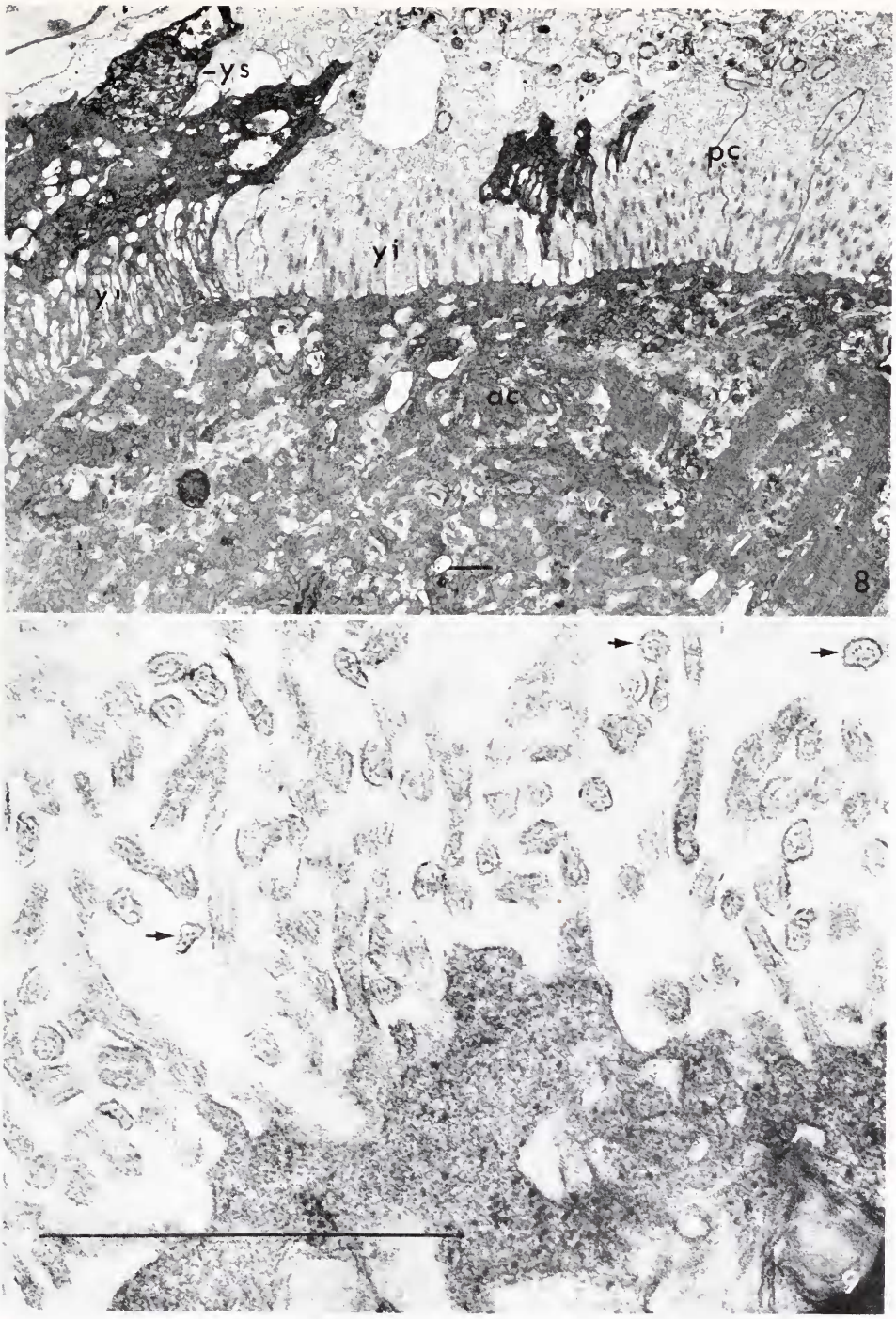


FIGURE 7. The ultrastructure shown in this electron micrograph quite clearly suggests the function of the accessory cell: absorption of nutriment for transport to the growing oöcyte. Microvilli absorb nutrients which are then transported or stored until required in the voluminous cisternae. Amorphous particles may represent processed material to be utilized by the egg; uranyl acetate, lead citrate: 91,140×; scale = 1 μ.



FIGURES 8 and 9. Many microvilli extend into parenchymal cells and remnants of accessory parenchymal cells, now changed to yolk spherules (Fig. 8). The microvilli in Figure 9 are free of enveloping cytoplasm. Arrows point to transverse sections which show distinct profiles of microvillar filaments; uranyl acetate; Figure 8, 5468×; Figure 9, 58,800×; scale = 1 μ.

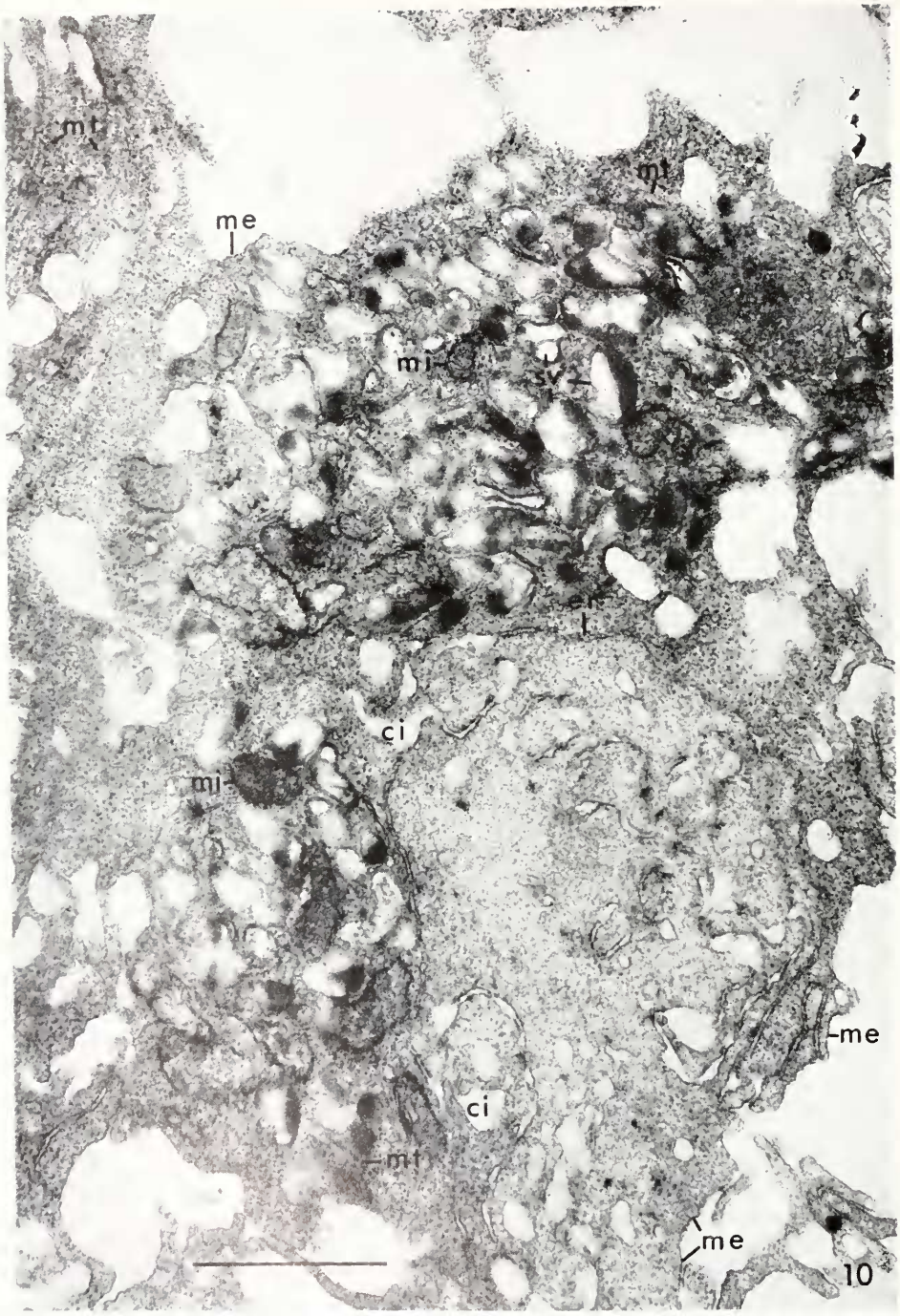


FIGURE 10. A single yolk spherule is composed of cellular elements without nucleus. Linear densities at surface suggest that the yolk spherule is membrane bound; uranyl acetate; 26,871×; me, membrane; mt, microtuble; sv, spherule vesicle; scale = 1 μ.

5. The yolk halo consists of two layers, each of different origin. The inner layer is composed of the numerous microvilli which extend from the accessory cell into membrane-delimited canals within cells of the turbellarian parenchyma. The spherules of the outer portion of the yolk halo are derived from accessory parenchymal cells. The function of the yolk-halo spherules is unknown.

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